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Improvement of Morphological Attributes and Fatty Acid Enrichment through Phyto-mediated Selenium Nanoparticles in *Brassica napus* L. Germplasm

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Abstract

The current research aimed to assess the potential impact of nano-selenium mediated by *Allium sativum* on the morphological attributes and fatty acid profiling of seven different *B. napus* germplasms. Various dosages, such as (T1;10, T2;20, T3;30, T4;40 and T5;50 mg/L) of nano-selenium, were applied at three different developmental stages, *i.e.*, (three-foliar stage, flowering stage and the fruiting stage. The results of UV-visible spectroscopy showed a peak wavelength of 291 nm, while FTIR shows different functional group alignments at a particular wavelength. The SEM validates the results about spherical nature of nano-selenium. The nano-selenium has shown significant variability in plant height, leaf area and number of siliquae, and less variation for stem diameter and 100-seed weight. The highest (173 ± 4 cm) plant height was recorded for Bn 23697 and the lowest was 96.67 ± 11.02 cm for Bn 23635 at 40 mg/L. The maximum stem diameter recorded was 7.36 ± 1.02 cm at T1 for Narc-Sarson, while the minimum was 3.86 ± 0.513 at T3 for Bn 23633. Considerable variation in leaf area was observed, with the lowest value 48.23 ± 6.18 cm^2 recorded in Super canola at T1 and the highest value 135.5 ± 23.6 cm^2 in Bn1605 germplasm under higher-dosage T5 treatment. The number of pods per plant is varied between 46.33 ± 10.41 for Bn 23697 at 20 mg/L and 12 ± 1.73 for Bn 23633 at T3 and T1 as compared to untreated. Seed weight varied among the varieties, with Super-Canola having the highest at 0.435 ± 0.007 g at T2, while Bn 23635 and Bn 23636 had the lowest at 0.305 ± 0.007 g at T3. Fatty acids, particularly oleic acid (OA), and proteins exhibit a weak positive correlation of 0.2. In contrast, erucic acid (EA) and oil content percentages showed a negative correlation (r = - 0.1). No correlation (r = 0) was observed between oleic acid and linolenic acid. The applications of nanoparticles in agriculture offer promising potential for modulating fatty acid profiles in oilseed crops. To fully elucidate these mechanisms, integrated transcriptomic studies are crucial to investigate nanoparticle-induced changes in gene expression.

Keywords: SeNPs (Selenium nanoparticles); Fatty acids profiling; *Brassica napus*; Plant morphology; Nanotechnology

Introduction

Nanotechnology has enabled agro-technological advancement and sustainable farming, revolutionizing both agriculture and ensuring global food security. A rising world population projection indicates that by 2050, the global food consumption will need to increase from 59% to 98% (Duro *et al.* 2020; Zaim *et al.* 2023). Consequently, a significant shift toward enhancing agricultural production is imperative. The use of nano-modified products in agriculture can hasten ongoing efforts to develop sustainable agriculture, sufficient food production and source of income for local farmers (Ahmad *et al.* 2023). Pakistan produces only 11% of the required edible oil; the remaining 89% is imported at a cost of approximately $3.419 billion. This heavy dependence on oilseed imports puts a strain on revenue and contributes significantly to the increase in edible oil prices in the country (Haggblade *et al.* 2010). A majority of the global population resides in impoverished rural regions that lack efficient agricultural expansion (Haggblade *et al.* 2010).

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Plant extracts can be used to synthesize green nanoparticles (NPs) because they are easy, expedient, ecologically benign and less time-consuming (Nasrollahzadeh *et al.* 2020). This green strategy is safe, simple to execute, ecologically friendly and reasonably priced (Zambonino *et al.* 2021). Nanoparticles (NPs) come in diverse sizes, shapes, compositions, and surface structures, offering ways to improve the physical and biochemical traits of crucial crop plants (Ikram *et al.* 2021). Roots hairs and leaves are the crucial entrance pathways for nanoparticles into the plant's cellular system. Plants respond differently to nanoparticles in terms of growth and metabolic activity (He *et al.* 2016). NPs provide a new option for farmers for increasing agricultural productivity while protecting the environment. The application of these NPs enhances crop protection, plant nutrition, and food safety by mitigating risks, increasing productivity, and reducing contamination (Rahim *et al.* 2021). Untimely crop sowing significantly disrupts phenological stages such as tillering, flowering and grain filling; all of which depend on favorable weather conditions for optimal growth (Mandal *et al.* 2018). Late transplantation can delay dry matter accumulation, hinder tiller emergence, and reduce tillers quantity (Mandal *et al.* 2019, 2022).

Potentially toxic elements (PTEs) have garnered increasing global concern due to their impacts on food security and human health. Selenium (Se), a naturally occurring metalloid and essential micronutrient exists in two primary forms: inorganic (*e.g*., selenite and selenate) and organic (*e.g*., selenomethionine [SeMet] and selenocysteine [SeCys]), whereas plants typically uptake both forms (Zhang *et al.* 2023). Selenium nanofertilizers are widely used in agriculture to produce selenium-enriched food as a strategy for biofortification. Recent technological advancements have enabled crop biofortification through exogenous application of micronutrients to soil and plants (Gong *et al.* 2018). However, Naderi *et al.* (2021) reported that plants absorb only 5-30% of applied Se-fertilizers, leaving the remainder in the soil. This residual selenium poses significant environmental risks and may contribute to severe human health complications, including birth abnormalities, female sexual dysfunction, skin lesions, respiratory distress, hair and nail disorders). Selenium (Se) levels in soil vary globally, with soils containing less than 0.5 mg/kg classified as selenium-deficient (Mirlean *et al.* 2018). These levels differ significantly depending on soil type. For instance, average selenium concentrations in soil across United Kingdom and China range from 0.1 to 4 mg/kg. However, soils with Se levels exceeding 200 mg kg^{-1} can become phytotoxic, posing risks to plant health (Shahid *et al.* 2018). Selenium deficiency in human is linked to disorders such as Kashin-Beck and Keshan diseases. To mitigate these risks, Shahid *et al.* (2018) recommend a daily selenium intake of 50 μg for adults. Adequate selenium supports plant resilience by enhancing pigment synthesis, net photosynthetic ratio, gas exchange, osmo-protectant accumulation, and secondary metabolite production during photosynthesis (Bano *et al.* 2021; Lanza and Reis 2021). Employing nanoparticles in agriculture provides protection to the plants from stress condition such as salinity and high temperatures (Skalickova *et al.* 2017). Commercially available SeNPs were applied along with Cd, and it was found that SeNPs have the ability to improve the growth biomarkers of *Brassica napus* under induced Cd stress. Several studies indicated that SeNPs enhance fruit growth and quality in oilseed crops, cereals, and Solanaceae plants even amidst varying stress conditions (Qi *et al.* 2021).

Canola (*Brassica napus*) is a vital broadleaf cash crop of the Brassicaceae family grown mainly in cool climates. Edible oils from *Brassica* oilseed crops dominate the market, yet surplus in developed nations is redirecting focus to their industrial potential. The key quality of seed oils is defined by their primary fatty acid composition (Mielke 2018). Rapeseed seeds are vital source of several foodstuffs, including oil, flour, meal, and bakery products (Sohail *et al.* 2020). The crop is significant for its oil production and ranks third after palm and soybean (Sharma *et al.* 2012). Canola oil is known to have less than 2% erucic acid and 30 μmol/g of glucosinolates of seed meal (Haile *et al.* 2014). Erucic acid in therapeutic doses treats adrenoleukodystrophy (X-ALD), a genetic disorder causing nervous system damage due to fatty acid accumulation. It includes linoleic acid, valued for its nutritional benefits, and oleic acid, prized for its stability when used as cooking oil (Cartea *et al.* 2019). Previous studies on the impact of nanoparticles have predominately focused on staple crops, while oilseed crops particularly *Brassica* spp remain understudied. This research investigates the biochemical and metabolic changes induced by nano-selenium in *Brassica napus*, especially its effects on the oil quality and quantity, and fatty acid profiles. The study aimed to explore how *Allium sativum*-derived nano-selenium, applied at distinct growth stages influence the morphology and fatty acid profiles of canola (*Brassica napus*).

Material and Methods

Synthesis and characterization of *Allium sativum* mediated nano-Selenium

The synthesis of nano-selenium occurred in two steps including 1) Plant extract preparation and 2) Synthesis of nano-selenium. In first step, the cloves of *Allium sativum* were place in shade and left for few days to dry completely. Then prepared fine powdered of *Allium sativum* using electric grinder machine. A 25 g of fine powder was then added to flask containing 300mL of distilled water. The flask was placed on hotplate for one hour and kept the temperature at 200°C. The resulted plant extracted was filters twice with Whatman filter paper to get clear filtrate, which was stored at 4°C till further use.

Secondly, add 0.436 g of sodium selenite salt in 500 mL of distilled water to prepared 1 m*M* precursor salt solution, by keeping the temperature of hotplate at 250°C and magnetic stirrer at 300 rpm for nano-selenium preparation. To initiate the reduction of selenium salt into nano-selenium, continuously added pure extract into beaker containing precursor salt solution. The results of changes in colour of salt solution (colorless) to red brick colour indicated that the nano-selenium preparation is obtained. Further, centrifuged the nanoparticles solution at 10000 rpm and pellets of nano-selenium will be observed. The pellets were washed with 5 mL of methanol and oven-dried at 65°C for 24 h. The resulting nano-selenium powder was collected for subsequent experiments. UV-visible spectroscopy (Uv-752pc spectrophotometer, wavelength range 200-800 nm) confirmed nanoparticle formation, as reported by Prasad and Selvaraj (2014). Scanning electron microscopy (SEM; JEOL JSM5910, Japan) was used to analyze samples at magnifications ranging 2.3 nm to of 30,000x, following methods described by Javed and Mashwani (2020). Fourier Transform Infrared (FTIR) spectroscopy (Perkin-Elmer Spectrum, Akron, OH, USA) was performed with an IR wavelength range of 500 to 4000 cm^{-1}. To isolate nano-selenium the reaction mixture was centrifuged three to four times at 8,000 rpm for ten minutes. The *supernatant* was replaced with deionized water and the pellet was stored as a dried powder. Finally, dried nano-selenium was subjected to FTIR analysis using the potassium bromide (KBr) pelleting method at a 1:100 ratio.

Chemical Reaction: Sodium selenite $_{(s)}$ + plant extract $_{(l)}$ → Nano-Selenium $_{(s)}$.

Seed collection and experimental layout

Seeds of seven different hybrid canola varieties were collected from the national oilseed program at the National Agriculture Research Center Islamabad (NARC). These accessions/varieties are now under extensive research to make high-yielding new varieties of *Brassica napus*. Prior to germination, seeds were surface-sterilized with 0.5% sodium hypochlorite for ten minutes and washed repeatedly with double-distilled water. The experiment was conducted in NARC from (10 Oct 2022 to May 2023). Six different concentrations of nano-selenium containing (control, 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L) were selected and applied foliarly to checked potential impact on plant growth and fatty acids profiling of *Brassica napus* varieties. Therefore, these accessions were selected for the study to check the impact of nono-selenium on yield. Applications of nano-selenium were given at 3-different growth stages, such as the three-foliar stage, flowering stage and fruiting stage). Fig. 1 shows the effect of nano-selenium on different plant attributes and fatty acid composition of *Brassica napus.*

Impact of nano-selenium on morphological attributes

Three randomly selected plants from each treatment were harvested at physiological maturity (after grain filling) to measure the following parameters: plant height (using a ruler, cm), stem diameter, leaf area, silique count per plant, and 100-seed weight (determined with a digital scale).

Determination of protein content

The techniques of Hames *et al.* (2008) were primarily employed in order to measure the total protein in seed of *Brassica* species. 2 g of chopped seed was broken down in concentrated H_2SO_4, filtered, and diluted to make 250 mL of sample preparation. The protein content was then determined by employing ammonia distillation and titration methods with 2 *M* NaOH.

Determination of glucosinolate content

Smith *et al.* (1985) outlined a glucosinolate content determination method for oil samples. A mixture of 200 mg pulverized seeds, 2 mL 50 m*M* glycine NaOH buffer (pH 9.0), chloroform, 100 m*M* citrate buffer (pH 5.0), and 10% chlorhexidine diacetate was incubated for 10 min. After introducing 200 mg of activated charcoal, a diastix strip measured color reflectance in the supernatant using a pre-calibrated TRUBLUGLU meter after 2 min.

Determination of fatty acid profiling

Oil was extracted from seeds of several *B. napus* germplasms using a Soxhlet apparatus (Aram *et al.* 2021). Oil content was measured by soaking 30 g of powdered seeds in 200 mL of hexane for six hours. Fatty acid profiles were analyzed using a gas chromatography system (Unicam 4600) to quantify oleic acid, erucic acid and linolenic acid. The analysis utilized a capillary column (30 m × 0.22 mm, 0.25 µm film thickness) with helium as the carrier gas at a flow rate of 1 mL min^{-1}). The column, injector port and detector temperatures were maintained at 180°C, 240°C, and 280°C, respectively. A 20 μL injection volume was used, and the percentage composition of each fatty acid was determined by comparing retention times between samples to standards under identical chromatographic conditions.

Data analysis

All the morphological parameters and fatty acid composition (analyzed in triplicate) were evaluated using a one-way ANOVA under a Complete Randomized Design (CRD). SPSS 16.0 was used to calculate the least significant difference (LSD) between means at *p*-value = 0.05. Letters denoting statistical significance were assigned using Minitab 19.1 for replicate data, where shared letters indicate no significant difference. R software was employed to generate heatmap and correlation matrix plot, and principal component analysis (PCA) was conducted using PAST 4.01.

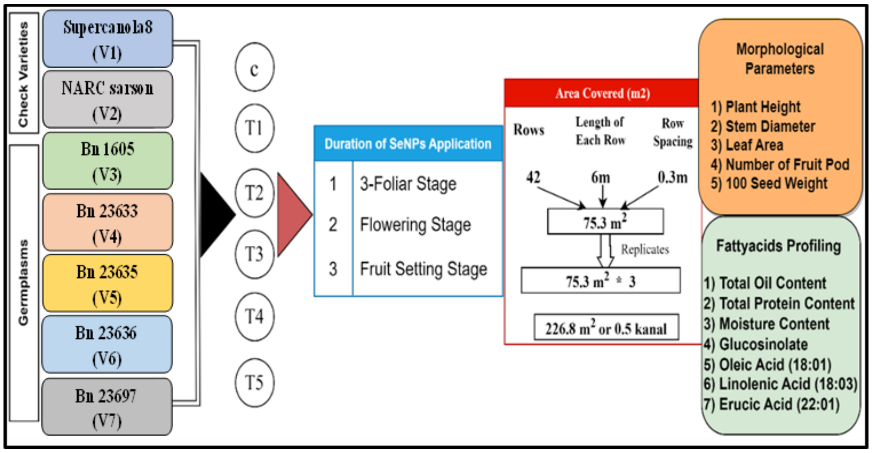

Fig. 1: Pictorial representation of experiment layout for *Brassica napus* varieties

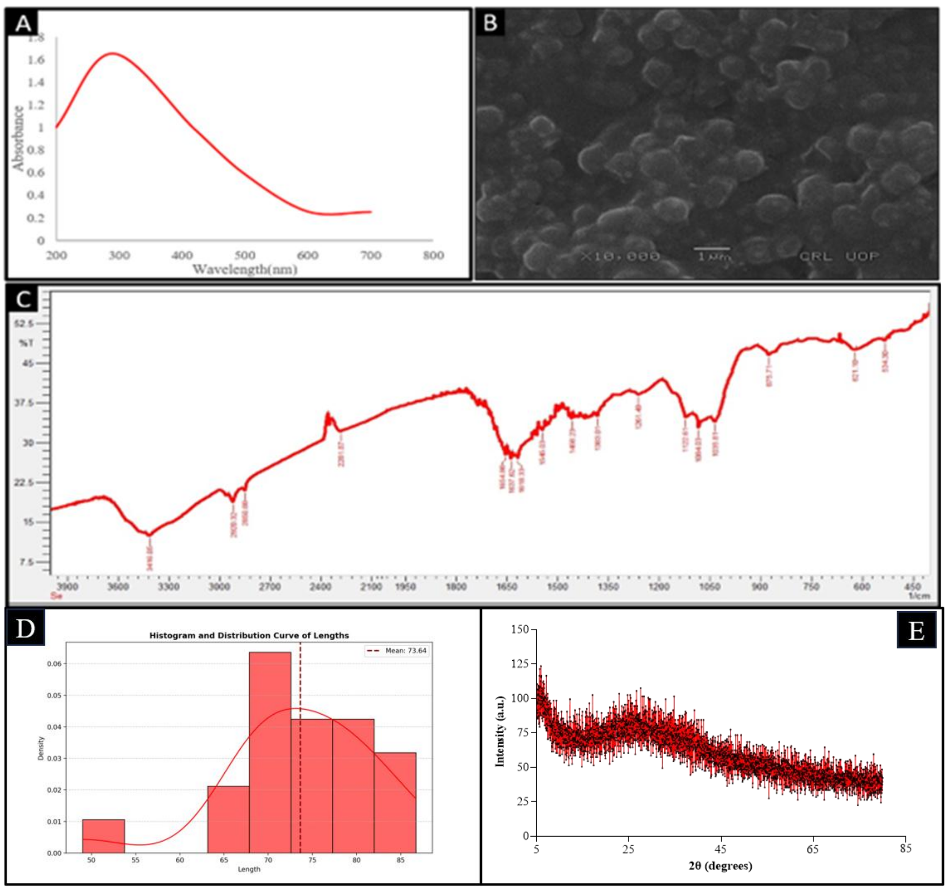

Fig. 2: (**A**) Uv-spectra of phyto-synthesize nano-selenium, (**B**) Scanning Electron Microscopy (SEM) for determination of structural morphology of NPs, (**C**) Fourier Transform Infrared (FTIR) revealed the associated functional groups in nano-selenium, (**D**) Histogram exhibited the average size of SeNPs, (**E**) X-Ray Diffraction determine the structural properties of phytosynthesized SeNP

Results

Characterization of phyto-synthesize nano-selenium

In this study, various characterization techniques were utilized for determination of nano-selenium, and spectral range confirms that UV-visible spectroscopy has the intense peak positioned at 291 nm (Fig. 2A). In this study, we have noticed that the nano-selenium synthesized from *Allium sativum* possessed an average size of 73.6 nm data provided by University of Peshawar (UOP), while setting the magnification power to 10,000 to clarify the spherical shape

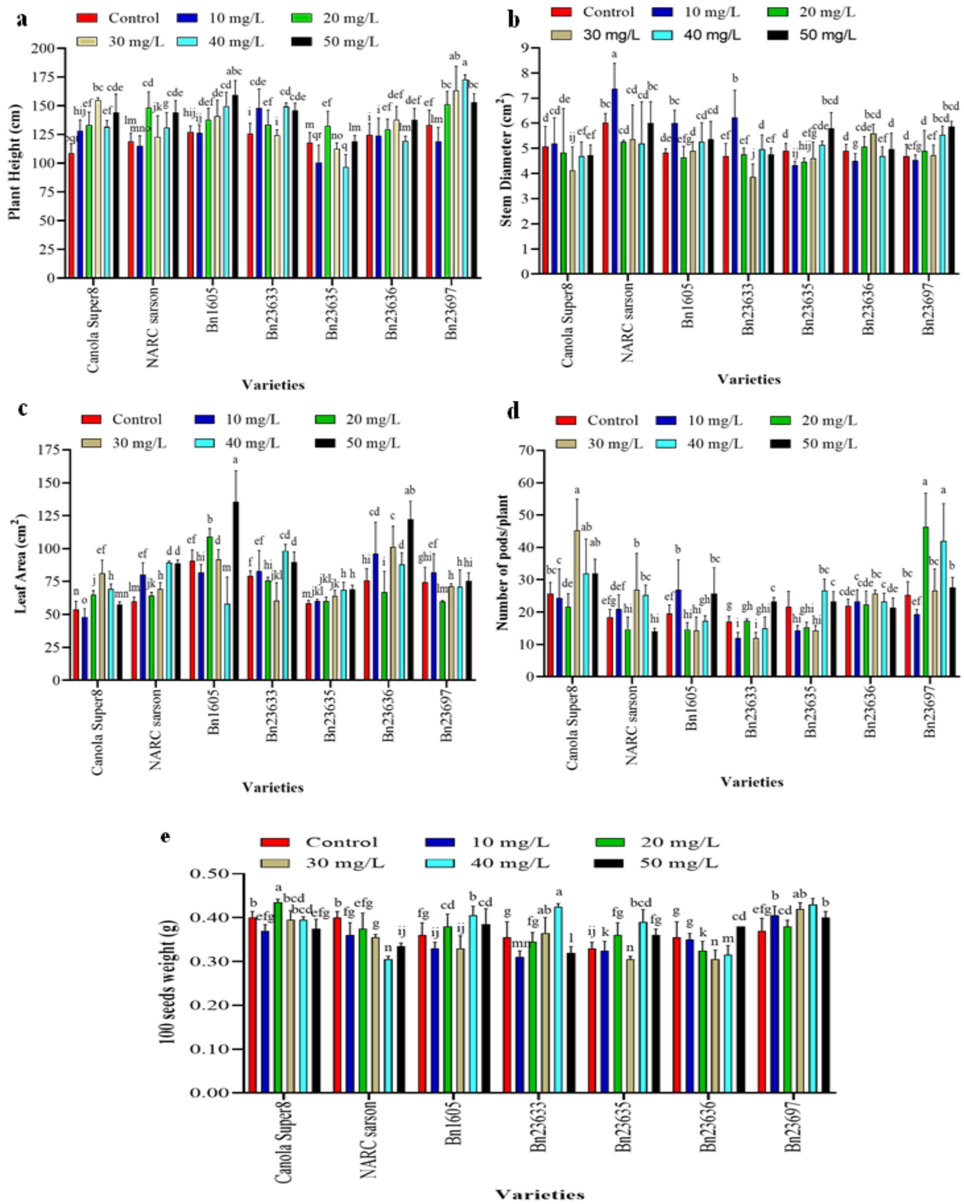

Fig. 3: Grouping information using the fisher LSD Method and 95% confidence for morphological attributes of *Brassica napus* germplasm influence by nano-selenium application. C+ represents for control plant, T1 (10 mg/L of SeNPs), T2 (20 mg/L of SeNPs), T3 (30 mg/L of SeNPs), T4 (40 mg/L of SeNPs) and T5 (50 mg/L of SeNPs)

for nano-selenium (Fig. 2B). Fourier Transform Infrared (FTIR) of nano-selenium determines the spectral ranges for various functional groups that are strongly involve in stabilization. It also provides crucial information related to peak shape, and peak intensity, as well as classifying the compound. The result indicates that seventeen distinct absorptions peaks at particular wavelengths were found, as shown in (Fig. 2C). Fig. 2 also shows that the peaks present are broad with weak intensity. To classify the compound class for various functional groups, results are given in detail, such as 534.30 nm for halogen group involvement in nano-stabilization. Additional peaks wavelengths corresponding to specific compound classes were identified as follow: 875.71 nm for (alkenes), 1064.03 (aromatic amines), 1261.40 (tertiary alcohol), 1363.01 (sulfonyl group), 2201.07 (esters), 2900.30 (Imine group), and 3498.05 for (carboxylic and Amide groups). While numerous studies have investigated FTIR analysis, their finding have provided limited insight into the correlation between spectral features and compound identification. In this study, we emphasized a systematic approach to map compound classes to their characteristics wavelength, while also analyzing peak morphology (shape) and intensity to enhance interpretative accuracy. The histogram (Fig. 2D)

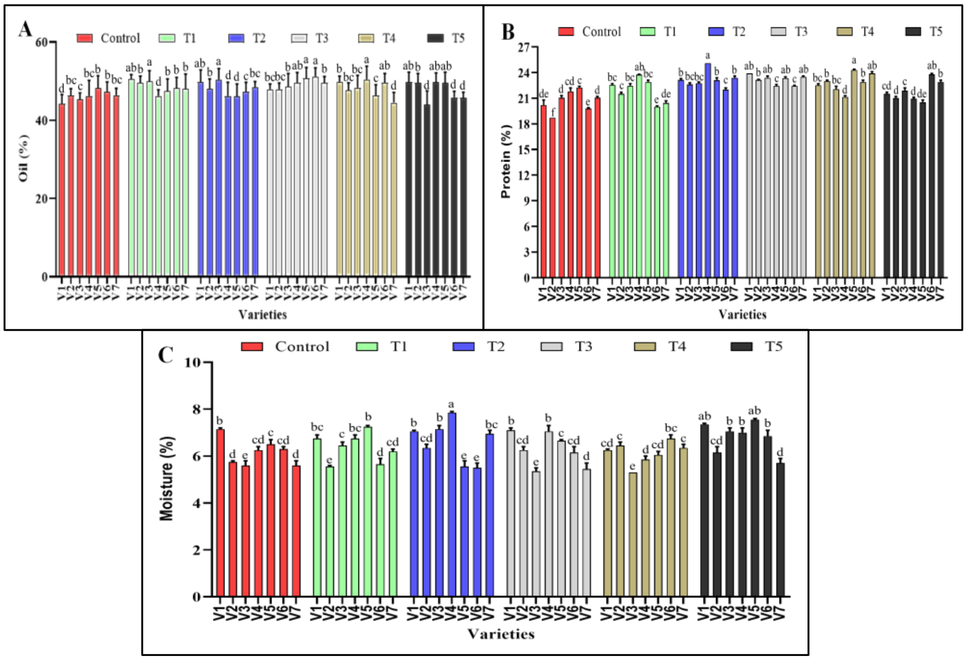

Fig. 4: Effect of *nano-selenium* on oil, protein and moisture content of *Brassica napus*. (**A**) Percentage oil composition, (**B**) Percentage protein content, (**C**) Percentage moisture content in seven different *B. napus* germplasm. V1; Supercanola8; V2; NARC sarson: V3; Bn 1605: V4; Bn 23633: V5; Bn 23635; V6; Bn 23636: V7; Bn 23697

reveals an average SeNPs size of 73.6 nm, as determined *via* ImageJ software. Fig. 2E displays distinct diffraction peaks 2θ angles, with intensities diminishing at higher angles (45°-85°), indicative of a crystalline structure in spherical selenium nanoparticles. The XRD pattern further shows a sharp shift in peaks intensities at 5° and 30°, with the most pronounced peak at 20°, confirming well-ordered crystallinity. Reduced intensities at higher angles suggest minor amorphous contributions to the nanoparticle structure.

Impact of nano-selenium on morphological attributes

The germplasm of *Brassica napus* exhibited significant variation in morphological attributes when treated with varying dosages of SeNPs. Fig. 3 highlights morphological attributes in details, in which the highest average plant height 173 ± 4cm was recorded in Bn23697, while the lowest 96.67 ± 11.02 cm occurred in Bn 23635 under the 40 mg/L SeNPs treatment (Fig. 3a). The correlation coefficient (R-Sq = 76.97%) indicated that foliar application of SeNPs significantly influenced plant height. Analysis of variance (ANOVA) of plant height across seven *Brassica napus* germplasms treated with SeNPs dosages yielded *F*-value of 6.84 ($P < 0.05$). Notably, exogenous SeNPs application had a marginal effect on stem diameter, with minimal variation observed among *Brassica napus* germplasms. Average stem diameter ranged from 3.867 ± 0.513 cm for (Bn23633 at 30 mg/L) to 7.367 ± 1.02 cm (NARC sarson T1 at 10 mg/L), as shown in Fig. 3b.

In same way, we measured leaf area (cm^2) in seven different canola (*Brassica napus*) germplasms. Super canola exhibited minimal changes in leaf area (48.23 ± 6.18 cm^2 at T1 treatment), whereas the Bn1605 germplasm showed the most pronounced response, with a significantly larger area of 135.5 ± 23.6 cm^2 under the high-dosage T5 treatment (Fig. 3c). ANOVA at a 0.05 significance level confirmed statistically significant differences in leaf area ($F = 6.96$, $R^2 = 87.17\%$). At maturity, pod/siliqua count per plant were recorded for all treatments (Fig. 3d). Bn 23697 produced the highest number of pods 46.33 ± 10.41 for Bn 23697 at 20 mg/L, while Bn23633 displayed the lowest count 12 ± 1.73 at T3 and T1, both relative to the control (Fig. 3d). We also measured 100-seed weights across treatments. Nano-selenium application during the flowering stage reduced seed shattering and minimized seed loss from siliquae in *Brassica napus.* Seed weights varied significantly, ranging from 0.305 ± 0.021 g for Bn23636 and Bn23635 at T3 to 0.435 ± 0.007 g Super Canola T2 (Fig. 3e).

Fatty acid profiling of *Brassica napus*

Seven germplasms differed significantly in fatty acid profiling. In the present study, nano-selenium mediated from *Allium sativum* was applied foliarly to access its impact on fatty acid composition. The average percentage of seed oil in seven different varieties of *Brassica napus* ranged from 44% to 51.2% of *B. napus*. Results indicated that the highest average oil content 51.2% was observed in BnV4T3 followed by BnV3T3 (50.70%), Super canolaT1 (50.50%),

Table 1: PC scores and Eigen value for different variable among seven *Brassica napus* germplasm

Sr. No	*Brassica napus* Seeds Parameters	PC Variance	Eigen value
1	Plant Height	18.79	401.57
2	Leaf Area	15.6	230.77
3	Number of Pods/Plants	12.2	174.03
4	100 Seeds Weight	11.59	56.45
5	Percentage Oil	9.9	38.39
6	Percentage Protein	7.4	9.68
7	Oleic Acid	6.4	3.28
8	Linolenic Acid	5.1	1.54
9	Erucic Acid	4.39	0.34
10	Glucosinolates	3.69	0.30

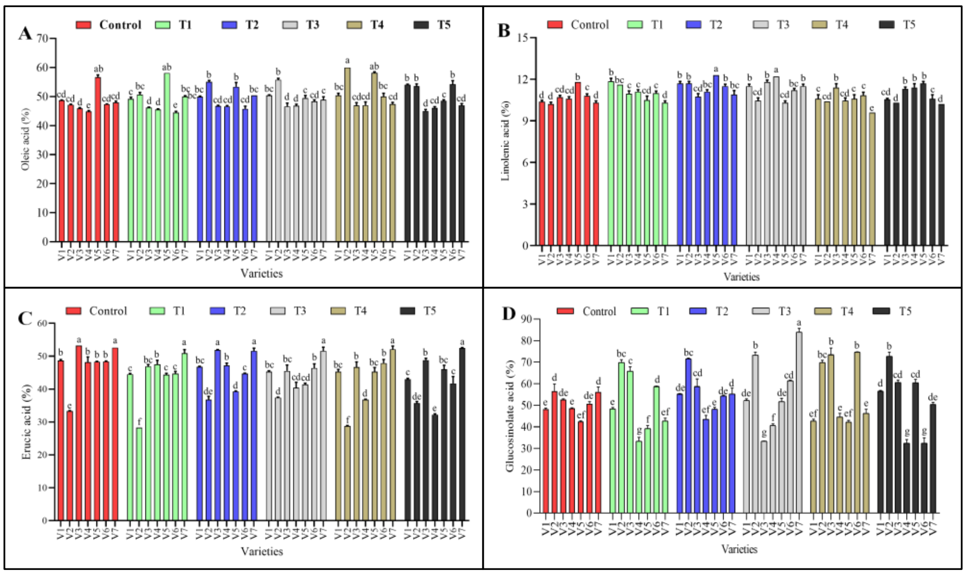

Fig. 5: Showing percent content of important fatty acid entities and glucosinolate content of *Brassica napus*, treated with plant mediated selenium nanoparticles. (**A**) Determination of percentage oleic acid, (**B**) Percentage of linolenic acid, (**C**) Erucic acid percent, (**D**) V1; Supercanola8; V2; NARC sarson: V3; Bn 1605: V4; Bn 23633: V5; Bn 23635; V6; Bn 23636: V7; Bn 23697

and BnV2T2 and BnV1T2 (both at 50.40%). The lowest average oil content 44% and 44.2% was recorded in BnV1T5 and Super canola (control), respectively. The percentage oil composition among seven different canola varieties at different nano-selenium concentrations is showed in Fig. 4A. In this study, protein content in seven *Brassica napus* germplasms ranged from 18.70 to 24.20%. Results demonstrated that the highest parentage of protein content 24.20% was observed in BnV3T5, followed by BnV2T1 (23.80%), BnV4T5 and BnV5T4 (23.70% each), and BnV5T2 (23.60%), respectively. NARC sarson (control) recorded the lowest protein content at 18.70% across all treatments (Fig. 4B).

In oilseed crop, grain moisture content is a crucial factor affecting seed longevity (Sohail *et al.* 2020). Study on different *B. napus* germplasms revealed significant variations in moisture content percentages when plants were treated with nano-selenium dosages (10, 20, 30, 40 and 50 mg/L). Result indicated moisture content values ranging from 5.30% to 7.90% across canola germplasms. The highest values 7.90% and 7.50% were observed in BnV2T2 and BnV3T5, respectively, following foliar nanoparticle application. Conversely, the lowest moisture content 5.30% and 5.50% was recorded in BnV1T3 and BnV1T4 among all treatments applied to *Brassica napus* (Fig. 4C).

The percentage composition of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in oil typically determines its acceptability for human consumption. As mentioned earlier, such compositions may raise concerns study might lead to cardiovascular diseases as the seed contains higher amount of saturated fatty acid constitution (Cartea *et al.* 2019). In this study, monounsaturated fatty acid (MUFA) such as oleic acid (18:01), erucic acid (22:01) and polyunsaturated fatty acid (PUFA) including linolenic acid (18:02) and glucosinolates content, were analyzed across various nano-selenium dosages as shown in (Fig. 5). Fluctuation in fatty acid profiles might be resultant from changing in climatic condition during crop cycle of *B. napus* germplasms or probably came from selenium nanoparticle application at three different growth stages. Marginal variation in oleic acid (18:01) was found which was shown in Fig. 5A. Result of present study demonstrated a greater variability in oleic acid content for different germplasm studied. Oleic acid with

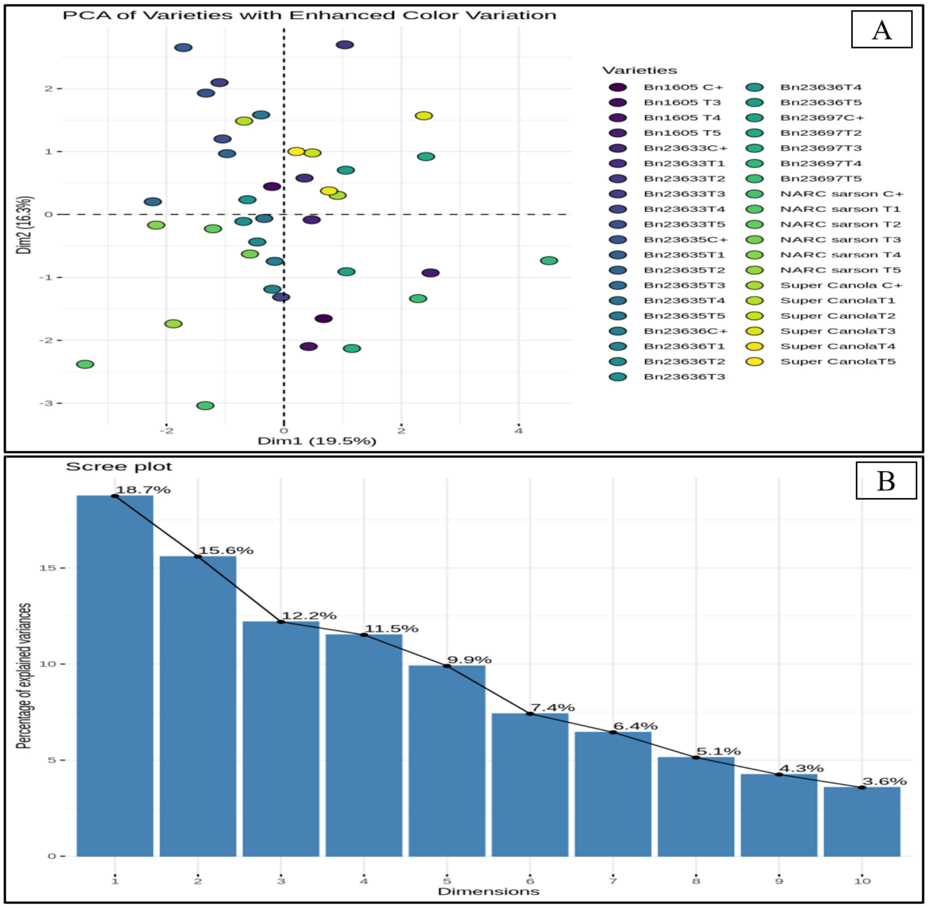

Fig. 6: (**A**) Principal Component Analysis (PCA) for 42 different treatments of nano-selenium among seven canola (*Brassica*) varieties. (**B**) Scree plot established percentage variance for fatty acids profile in canola

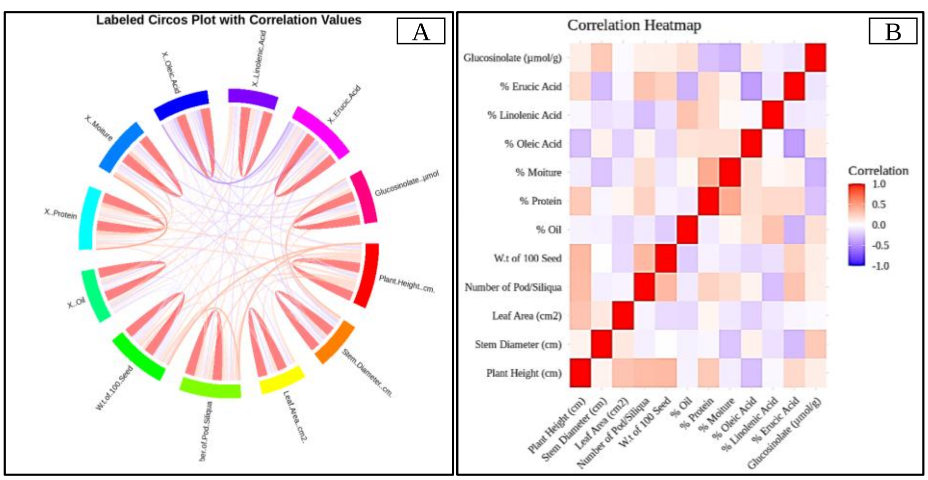

Fig. 7: (**A**) Correlation circos plot determine interaction between plant morphological and fatty acid parameters among seven different *Brassica napus* germplasm, (**B**) Heatmap constructed for morphological parameters and fatty acid composition

composition higher that 55% recorded for different dosages including 55.40% and 60% for (NARC sarsonT1 and T4), 57.2% and 58.10% for (BnV3control and T1), accordingly.

The bar graph 3.4 shows detailed results of fatty acid composition for all treatments among seven different canola germplasms. In a similar way, variation in percentage linolenic acid composition was recorded. It was found that nano-selenium foliar applications at various dosages including 10 mg/L (T1), 20 mg/L (T2), 30 mg/L (T3), 40 mg/L (T4), and 50 mg/L (T5), had greater linolenic acid percentage compared to untreated plants (control). The maximum 12.30% linolenic acid content in seeds was found for BnV3T2 and the lowest 9.60% was recorded in BnV5T4 (Fig. 5B). Furthermore, present study shows variation in erucic acid contents among different *Brassica napus* germplasms. The results demonstrated that erucic acid content ranges from 28.30% to 53.20% in NARC sarsonT1 and BnV1 untreated plant, respectively. The detail results of erucic acid composition were given in Fig. 5C. Finally, glucosinolates concentrations (*µ*mol/g) in *Brassica napus* under nano-selenium treatments were measured, as shown in Fig. 5D. The results demonstrated the highest concentration of 84 *µ*mol/g in BnV5T3, followed by 75.3 µmol/g for NARC sarsonT2 and 74.6 *µ*mol/g in BnV4T4. The lowest glucosinolates concentration (32.4 *µ*mol/g) was recorded for BnV4T5 and BnV2T5 (Fig. 5D).

Statistical result of protein, oil, moisture and fatty acids

Principal Component Analysis (PCA) was performed on seven different *Brassica napus* germplasms treated with varying dosages of nano-selenium. The PCA results, including eigen-values and percentage of variance explained for each variable, are presented in Table 1. All treatments clustered along specific axes based on shared or closely related characteristics. For instance, BnV3 (control), BnV5T2, BnV5T1, BnV3T4, BnV4T4, BnV5T5, BnV5T4, BnV5T3, Super canola (control), Super canolaT2 and Super canolaT4, which exhibit similar fatty acid composition profiles were position along positive X-axis and Y-axis (Fig. 6A). In contrast, the negative X-axis combined with positive Y-axis grouped treatments with common traits, including NARC Sarson T4, NARC Sarson T1, NARC Sarson T5, Bn V3T1, Bn V4T5 and Bn V3T2. Treatment located on opposing axis displayed distinct trait variability. Additionally, the scree plot in Fig. 6B illustrates the percentage variance attributed to each principal component. The major contributed principal components (PCs) for total variation (67.9%) included plant height, leaf area, number of pods/plants, 100 seed weight and oil percentage. Among all components, PC1 exhibited highest percentage variance of 18.79% and eigenvalue (401.57). PC 2 and PC 3 accounted for 15.6% and 12.2% of the variance, with eigenvalue of 230.77 and 174.03, respectively.

A correlation circos plot (Fig. 7A) was generated to visualize interactions between all variables, with red indicating positive correlations and blue representing negative correlations. Broader line denotes stronger positive correlations, while thinner line reflects weaker negative correlation. The analysis revealed a positive correlation between percentage protein content and oleic acid (OA). In contrast, percentage erucic acid exhibited a negative correlation with percentage protein across the studied germplasms. However, no significant correlation was observed between oleic acid and linolenic acid. Additionally, oil content in canola germplasms showed a negative correlation with erucic acid (EA). Detailed results for parameters such as moisture, oil, protein, oleic acid, linolenic acid, and erucic acid are provided in (Fig. 7A). Meanwhile, Fig. 7B illustrates a heatmap visually summarizing correlations between all variables across *Brassica napus* L. germplasms. Values closer to +1 indicate strong positive correlations, while those closer to -1 represent strong negative correlations; where near 0 indicate no correlation (Fig. 7B).

Discussion

The selected concentrations of Se NPs (10-50 mg/L) were based on preliminary trials and existing literature demonstrating dose-dependent responses in oilseeds crops and other plant species. Plant-based nanoparticles are typically applied to plant *via* two different methods: seed priming or foliar application. These interactions can enhance plant growth and productivity (Ahmad *et al.* 2024). Previous study confirms that SeNPs at concentrations below < 50 mg/L often improve plant growth, antioxidant activity, phytoconstituents levels and stress tolerance in crops, while higher concentrations may induce toxicity. Additionally, Wrobel *et al.* (2020a, b) demonstrated that selenium uptake in plants is strongly influenced by soil selenium concentrations. Ralphs (2002) identified plant species including woody asters, Astragalus and *Stanleya pinnata*, capable of accumulating selenium (Se) at levels exceeding 3000 mg. kg^{-1}, classifying them as Se hyperaccumulators. Such high concentrations can render these plants toxic to livestock when ingested. Bano *et al.* (2021) established threshold levels of Se in staple crops such as wheat, barley, and alfalfa, noting their ability to accumulate selenium at concentrations up to 50 mg. kg^{-1}. The garlic extract mediated selenium nanoparticles (Se NPs) significantly improve morphology, antioxidant status and metabolic profiles in sesame under induced biotic stress (Ahmad *et al.* (2023). Similarly, Iqbal *et al.* (2019) investigated the impact of green-synthesized silver nanoparticles (Ag NPs) on wheat under heat stress. They observed that heat stress reduced plant fresh and dry mass, root length, shoot length by 0.02%, 0.16%, 2.5% and 6.2% respectively. In contrast, silver nanoparticles application enhanced root length, shoot length and fresh weight by 5.4%, 26.1% and 2%, respectively. Seed weight, a physical indicator of seed quality directly influences yield. Additionally, sowing practices critically affect the nutritional quality of subsequent plants, making this an essential agronomic consideration. Hafeez *et al.* (2015) reported that increasing zinc nanoparticles (Zn NPs) dosages in spinach adversely affected growth parameters such as seed weight, pod number and stem diameter. Conversely, zinc and copper nanoparticles have been extensively studied and shown to enhance crop morphology and physiology under specific conditions. The highest fresh and dry weight of basil aerial parts was observed with the application of Zn and Cu nanoparticles at dosages of 4000 mg. kg^{-1} and 2000 mg. kg^{-1}, respectively. Similarly, fresh flower weight and shoot weight were also highest in these treatments, suggesting that combined nanoparticle applications exert a pronounced effect on the plant growth. Comparable improvements were noticed in chlorophyll content, biochemical parameters and antioxidant status, as reported by (Abbasifar *et al.* 2020). In this study, analogous results were observed with lower nano-selenium dosages applied to *B. napus* germplasm, which are unlikely to induce toxicity in quantitative morphological traits. However, higher nanoparticle dosages may lead to adverse effects such as abnormal gynoecia, flower abortion, pre-mature silique opening, key factors contributing to reduced canola yield.

In future plant breeding programs, heterogeneity in oil content is an astonishing feature that positions it is a potential asset for exploitation. Gheshlaghi *et al.* (2019) discovered that the application of ZnONPs at 100 mg/L increase oil content in seeds compared to untreated plants. Another study by (Sharafi *et al.* 2015) revealed that the *Brassica napus* accessions varies from from 36% in (Slm046) to 56% (Okapi). In accordance with previous findings, cultivated rapeseed (*B. napus*) produces higher seed oil than wild rapeseed, as suggested by Sohail *et al.* 2020. Fig. 4B illustrates the percentage protein content among seven different *B. napus* germplasms under varying doses of nano-selenium. Comparable results were by Tang *et al.* (2019), who determine the protein content in canola accessions ranges between 19.77% and 30.11%. Oil quality is influenced by rapeseed fat and protein content, which are affected by agrotechnical practices, environmental conditions, and varietal traits, as described by (Barthet 2016). Similarly, Tiwari *et al.* (2021) stated that protein content variability in *Brassica napus* largely depends on overexpression of genes controlling the trait. Meanwhile, nanoparticle that shown lowest moisture content is considered to be more respectable in term of resistivity against fungal attacks. Prior research by Rokosik *et al.* (2019) aligns with these findings, concluding that canola genotypes with genotypes with moisture content up to 7% exhibit consistent results.

This work will help identify the potential impact of nano-selenium mediated from *Allium sativa* on total protein content in seeds of *Brassica napus* germplasm. Our findings contradict previous studies by other authors. The pervious study of Sagan *et al.* (2019) reported an average oleic acid content of 17 % in response to copper nanoparticles (Cu NPs) treatment, which is significantly lower than the values observed in the present study. Inconsistencies were also observed for linolenic acid levels in canola germplasm when compared to earlier reports by (Asif 2011; Wang *et al.* 2018; Kachel *et al.* 2023). Unlike this study, prior research did not apply nanoparticle treatments to canola germplasm but instead focused on evaluating genetic, phenotypic and fatty acid variation in response to environmental conditions. A study by Ahmad *et al.* (2023) demonstrated that the seed priming and foliar treatment of sesame with Se NPs significantly improves fatty acid composition. The application of Se NPs at 40 mg/L is substantially higher levels of tocopherol, sesamin and sesamol as observed through GC-MS analysis. These compounds were linked to the elicitation of unsaturated fatty acids, including oleic and linoleic acids, *via* KEGG pathway analysis and metabolite set enrichment analysis (MSEA) (Ahmad *et al.* 2023). Similarly, Sohail *et al.* (2020) reported that treating canola with zinc nanoparticles (MNPsat 40 mg/L and 90 mg/L increased oleic acid and linolenic acid levels. In this study, erucic acid composition was analyzed, and the results align with finding reported by Zafar *et al.* (2020), who observed significant variability in high erucic acid levels would require targeting the genes regulating these traits, as metal nanoparticles alone do not inhibit erucic acid accumulation in canola seeds. In addition, glucosinolate content showed strong alignment with earlier findings by Zafar *et al.* (2020). Minor variations in glucosinolate levels between studies may stem for different in the germplasm used.

Conclusion

This study focused on assessing the effects of nano-selenium on the morphological and fatty acids profiles of *Brassica napus* germplasms. The finding revealed that lower concentrations of nano-selenium (*e.g*., 30 and 40 mg/L) can enhance growth parameters and improve fatty acid profiles. However, higher doses, such as 50 mg/L, may induce plant stress, leading to reduced growth and biological yield. Our results indicate that nanoparticle application significantly influences plant height, number of siliquas, leaf area but has a non-significant impact on stem diameter and seed weight. The application of 30 mg/L nano-selenium resultant in a 42% increase in plant height, 76% increase in number of pods per plant in Super canola and a 14.2% increase in stem diameter (Bn 23636). At 40 mg/L, the treatment showed a maximum 19% increase in plant height (Bn 23633), while 50 mg/L nano-selenium led to a 49% increase in leaf area (Bn 1605). These findings suggest that exogenous nano-selenium application may enhance morphological diversity and fatty acid composition, offering a potential strategy to boost oil content and optimize fatty acid profiles. While prior study suggested the variability in fatty acid composition to climatic conditions, this study highlights the broader implications of nano-selenium in mediating these traits. By correlating nanoparticle dosage with morphological and biochemical outcomes, our work expands the understanding of nano-selenium role in crop improvement. Furthermore, we observed a positive correlation between protein and oleic acid content, whereas erucic acid exhibited an inverse relationship. The increase in oleic acid production in canola germplasm appears directly linked to reduction of erucic acid and shows limited association with linolenic acid levels. As an oilseed crop, *B. napus* remains a focal point for breeders aiming to address gaps in optimizing fatty acid composition. These finding hold promise for genetic studies, including the re-synthesis of *B. napus* as genotypic and phenotypic correlations align with the biosynthetic pathways of major fatty acids.

In conclusion, our study demonstrates that nano-selenium application significantly influences fatty acid composition of *Brassica napus* germplasms. The growing use of nanotechnology in agriculture and other sectors of the global economy have raised concerns about potential risks to human and environmental health. Manufactured nanoparticles can be released intentionally or accidentally into the environment through pathways such as atmospheric emissions, industrial waste streams, or consumer products. Furthermore, nanomaterials incorporated into paints, textiles, and cosmetics can accumulate in ecosystems proportionally to their production and usage rates. Once released, these nanoparticles can settle on terrestrial and aquatic surfaces, contaminating soil and infiltrating surface and groundwater sources. Additionally, wind erosion, rainwater runoff, or accidental spills can transport nanoparticles from landfills, wastewater, or industrial sites into aquatic systems. While nanoparticles, show promise for enhancing crop productivity, their environmental hazards, such as bioaccumulation disruptions must also be rigorously studied, documented and mitigated to ensure sustainable agricultural practices.

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Author's Contribution

ZY participated in document analysis and manuscript writing, Ubaidur Rahman participated in data collection, IA participated in data analysis and literature review, HK contributed in review and editing, MK participated in data analysis and software, ZURM participated in research design. All the authors read and approved the final manuscript for publication.

Conflict of Interest

The authors declared no conflict of interest.

Data Availability

Data supporting this study are available from corresponding authors upon reasonable request.

Ethics Approval

Not applicable to this paper.

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